First High-Resolution Structure for an Aquaporin

Glycerol Conducting Channel Reveals Selectivity Mechanism

How can a modest molecule be a great bouncer? Like a heavy at an exclusive night club, an aquaporin can see to it that only the desired molecules enter a cell, stiffly excluding all others. Yet, despite their finickiness, these proteins allow rapid influx of molecules of the right class. Aquaporins are a large family of proteins that selectively yet efficiently transport water or glycerol molecules—but no ions—across membranes in plants, animals, bacteria, and even yeast. Ten of these occur in humans, and more than 150 have been sequenced. But until now, scientists have not had a clear enough view of their structure to understand just how they enforce their exclusivity.

A group of researchers from the University of California, San Francisco, has solved the structure of GlpF, a glycerol-conducting member of the aquaporin family found in *Escherichia coli*, to a resolution of 2.2 Å. This first high-resolution structure of an aquaporin reveals the mechanism for GlpF's selectivity. Because of the close sequence homology among the members of the aquaporin family, this discovery may have far-reaching implications for the function of all the aquaporins.

The researchers used the Macromolecular Crystallography Facility at Beamline 5.0.2 to solve the structure of GlpF, crystallized with three glycerol molecules conveniently passing through each of four channels. Their model was based on multiple isomorphous replacement and anomalous dispersion studies, yielding R_{cryst} and R_{free} values of 19.7 and 22.3, respectively.

The overall structure of GlpF comprises four parallel channels arranged with fourfold symmetry about a central axis that runs perpendicular to the membrane bilayer. Each channel is formed by six membrane-spanning alpha helices and two half-membrane-spanning alpha helices that also form a hydrophobic outer shell. The complete molecule's amino acid sequence can be divided into two halves that show about 20% homology, each containing an arginine-proline-alanine (NPA) sequence near its center. In the three-dimensional structure, the two halves are related by a quasi-twofold axis running through the center of the bilayer, perpendicular to the fourfold axis. A tight link between the two was found to be formed by the NPA sequences, the proline ring of each being clasped between the other's proline and neighboring alanine residues.

The structure of the channel revealed much about GlpF's selectivity mechanism. A constriction just wide enough to accommodate linear alditols such as glycerol in single file begins near the quasi-twofold axis, 8 Å toward the periplasmic side (the side nearer the outside of the cell) and ends on the cytoplasmic surface. Inside this "selectivity filter," a hydrophobic corner is formed by the rings of a tryptophan and a phenylalanine residue. The carbon backbones of the glycerol molecules become wedged against this corner so that their oxygens come near residues with which they can form successive hydrogen bonds. Thus, passage through the channel depends not only on narrowness but also on a molecule's ability to become polarized along the correct axis. Because some of the successive hydrogen-bonding residues are hydrogen bond donors and others are acceptors, the molecule passing through must have atoms that can act both as acceptors and as donors. This criterion facilitates the passage of glycerol but leaves ions grumbling at the door. ■

Robert M. Stroud (stroud@msg.ucsf.edu), University of California, San Francisco.

D. Fu, A. Libson, L.J.W. Miercke, C. Weitzman, P. Nollert, J. Krucinski, and R.M. Stroud, "Structure of a glycerol-conducting channel and the basis for its selectivity," *Science* **290**, 481–486 (2000).

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FIRST HIGH-RESOLUTION STRUCTURE FOR AN AQUAPORIN

Glycerol Conducting Channel Reveals Selectivity Mechanism

- **Aquaporins: conducting channels for water or glycerol with exclusion of all ions**
- **First high-resolution structure of an aquaporin**
  - *Obtained at Macromolecular Crystallography Facility, Beamline 5.0.2*
  - *Method: multiple isomorphous replacement and anomalous dispersion*
  - *Resolution: 2.2 Å*
  - *Crystal:* Escherichia coli *glycerol facilitator (GlpF) with glycerol molecules*
- **Narrow channels exclude molecules wider than linear alditols**
- **"Selectivity filter" in each channel**
  - *Alkyl backbone of glycerol molecule wedged against hydrophobic corner*
  - *Hydrogen bonding between glycerol molecule and GlpF residues across channel*
- **Molecules passing through must be correct size, polarizable along correct axis**

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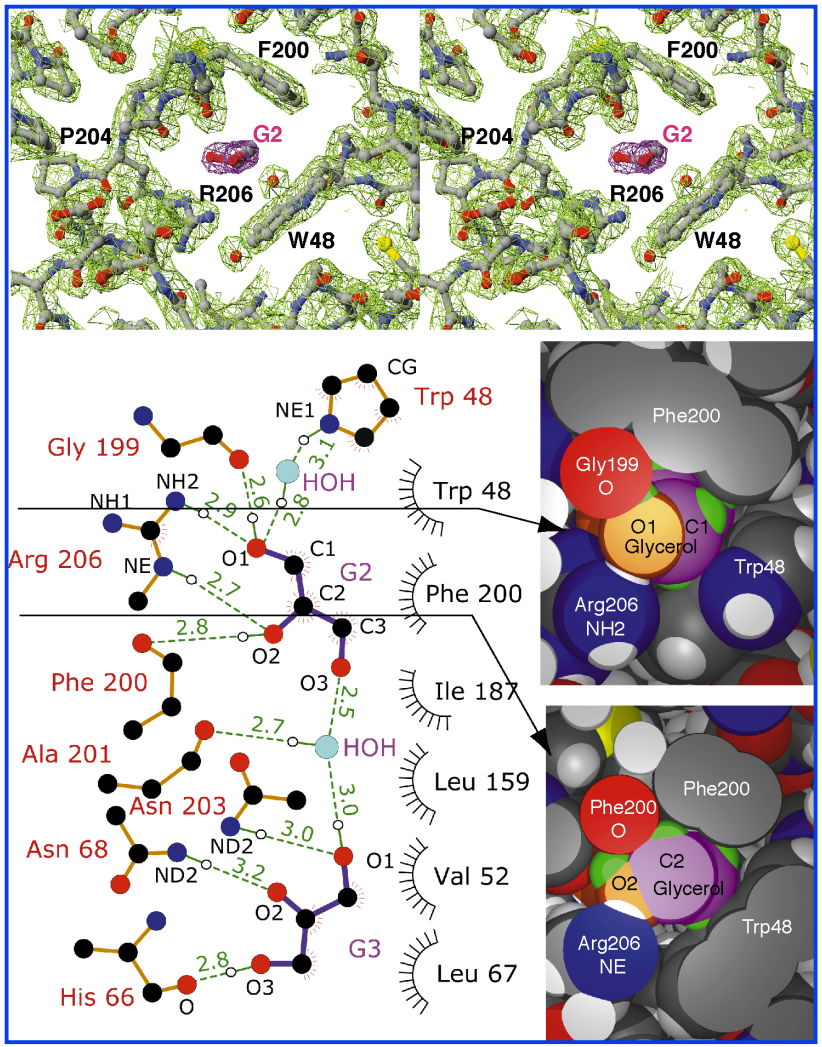

Top, stereo view of electron densities, looking down one channel of a GlpF molecule. A glycerol molecule (G2) is wedged between a phenylalanine residue (F200) and a tryptophan residue (W48). Nearby arginine (R206) acts as a hydrogen bond donor. Below, left, is a view along the length of the "selectivity filter," showing the hydrogen bonding (dotted lines). Radial lines indicate hydrophobic areas. The arrows indicate the cross sections through the channel, shown at right.

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